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# Lysosomal Membrane Glycoproteins: Properties of LAMP-1 and LAMP-2

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FOOTNOTES

Abbreviations used: LAMP, Lysosome Associated Membrane Protein; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PBS, phosphate buffered saline; HBSS, Hank's Buffered Salt Solution; NP-40, Nonidet-P40; BSA, Bovine Serum Albumin, FBS, Fetal Bovine Serum.

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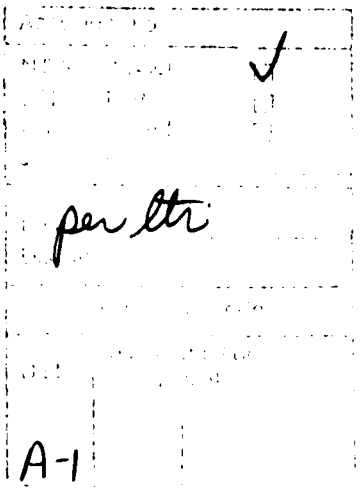


ABSTRACT

Several properties of the lysosomal membrane glycoproteins LAMP-1 and LAMP-2 have been analyzed. Each molecule was strongly associated with lysosome membranes and was extracted only in the presence of detergent. Studies of the biosynthesis and processing of the glycoproteins showed that each contained a polypeptide core of approximately 43,000 daltons as identified by use of tunicamycin and endoglycosidase H. Nascent glycoproteins pulse-labeled for 5 min with [$^{35}$S]methionine were approximately 92,000 daltons. These precursor molecules were processed in 30 min to highly heterogenous mature glycoproteins of approximately 110,000 daltons (LAMP-1) and 105,000 daltons (LAMP-2). Concomitant with the increase in apparent molecular weight the molecules became endoglycosidase H resistant and acquired sialic acid residues, indicating that they were converted to complex-type oligosaccharides. The final maturation of the glycoproteins was blocked by monensin. Immunohistochemical analysis of tissues from Balb/c and Beige/J mice showed that the molecules were present on many types of cells, consistent with their presence in lysosomes. The patterns of tissue expression of LAMP-1 and LAMP-2 in the two mouse strains were the same except that the intensity of staining of LAMP-2 was less than that of LAMP-1. LAMP-2, but not LAMP-1, gave a decreased immunofluorescent staining intensity in transformed HaNIH as compared to NIH/3T3 cells. The marked similarities between the LAMP proteins raise the consideration of common functions, possibly associated with the high oligosaccharide content of the molecules.

## INTRODUCTION

Lysosomes have a central role in cellular homeostasis as sites for digestion of foreign materials and for degradation of intracellular components undergoing autolytic processing (de Duve, 1983). Recently, several reports have described glycoproteins associated with lysosomal membranes. These molecules are of interest for their possible role in the biogenesis of lysosomes or in certain specialized functions, such as fusion with other vesicles, selective recognition and transport of molecules, vesicle acidification, and resistance to lysosomal hydrolytic enzymes.

We previously identified two glycoproteins of mouse cells specifically localized in the lysosomal membrane, LAMP-1 of 105,000 to 115,000 daltons and LAMP-2 of 100,000 to 110,000 daltons (Chen et al., 1985a; Chen et al., 1985b). Electron microscopy with ferritin bridge labeling showed that both were localized just beneath the limiting lysosomal membrane of large "dense body" lysosomes and smaller multivesicular lysosomes. LAMP-1 and LAMP-2 appeared to be different polypeptides as indicated by tryptic peptide mapping, sequential immunoprecipitation (Chen et al., 1985a), and N-terminal amino acid sequence analysis (Chen, unpublished). Additional studies of LAMP-1 suggested that the molecule contained a large number of N-linked oligosaccharides as the glycoprotein pulse-labeled with [$^{35}$S]methionine and treated with endoglycosidase H yielded a core polypeptide of 45,000-daltons (Chen et al., 1985b).

LAMP-1 and LAMP-2 were compared with other recently described lysosomal membrane glycoproteins. Reggio et al., (1984) and Tougard et al., (1985) identified a protein of about 100,000 daltons present both in lysosomes and prelysosomal acidic vesicles. Polyclonal antibodies against this protein reacted with a gastric mucosal $H^+/K^+$ATPase and it was suggested that the protein may be a component of the proton pump involved in vesicle acidification. Lewis et al., (1985) described glycoproteins of 120,000,

100,000, and 80,000 daltons located in lysosome membranes. The 120,000-dalton component was found to contain a 42,000-dalton polypeptide core and at least 18 N-linked oligosaccharides rich in sialic acid. In addition, Lippincott-Schwartz and Fambrough (personal communication) have identified a glycoprotein of approximately 100,000 daltons in chick embryo fibroblasts that yielded a core polypeptide of 48,000 daltons; this molecule was located predominantly in lysosomal membranes with smaller fractions present in endosomes, the Golgi apparatus and the plasma membrane.

In this report we describe the further characterization of LAMP-1 and LAMP-2. The molecules were very similar in their biosynthesis and expression in vivo and both contained a large number of N-linked, highly sialiated oligosaccharide chains. The similarity in oligosaccharide composition of these glycoproteins and those described by Lewis et al. (1985) and Lippincott-Schwartz and Fambrough (1985) raise the possibility of common functions that could be related to the biogenesis or function of lysosomes.

## MATERIALS AND METHODS

### Antibodies

Monoclonal antibody anti-LAMP-1, IgG2a, was derived from spleen cells of a rat immunized with a membrane fraction of mouse embryo 3T3 cells (Hughes and August, 1981). Monoclonal antibody anti-LAMP-2, IgG2a, was derived from spleen cells of a rat immunized with a lentil lectin membrane fraction of Balb/c 3T3 cells (Chen et al., 1985a).

### Cells

NIH 3T3 cells (Jainchill et al., 1969) were obtained from Dr. Don Blair of the Frederick Cancer Institute, MD and were used at passage 4. HaNIH cells (Scolnick and Parks, 1974) and P3e8D1 (Shevach et al., 1972) were obtained as previously described (Hughes and August, 1981) and maintained as described (Chen et al., 1985b).

### Biosynthetic labeling of Cells

HaNIH cells grown to 80-90 percent confluence in 75 $cm^2$ or 150 cm flasks were washed with warm Hank's Buffered Salt Solution (HBSS) (Gibco, Chicago Falls, Ohio), and cultured with 6 mls of methionine-free media (Gibco) for 2 h at 37°C. The cells were then pulse-labeled with 6 mls of labeling media [methionine-free media supplemented with 125 μCi/ml of [$^{35}$S]methionine (Amersham, Arlington Heights, IL)] for 5 min or as indicated in the text. Cells were then washed two times with warm HBSS and collected immediately or incubated with Dulbecco's minimal essential medium containing 10 percent fetal bovine serum for the times indicated in the text. The inhibitors tunicamycin or monesin, when present as indicated in the text, were added with each of the media. Cells to be harvested were washed three times with PBS at 4°C, removed from the plate by scraping, and collected by centrifugation.

Cell Extraction and Protein Immunoprecipitation

Metabolically labeled cells were extracted with a lysis buffer [10 mM Tris-HCL, pH 7.5, 0.5 percent Nonidet-P40 (NP-40, Particle Data Inc., Elmhurst, IL), 5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and 0.15 M NaCl (Hughes and August, 1982)]. After 30 min on ice, the lysate was freeze-thawed three times, and the detergent-insoluble material was removed by centrifugation at 100,000 x g for 60 min at 4°C. The soluble extract containing $1\text{-}5X10^6$ acid precipitable dpm was incubated with 200 µl monoclonal antibody from tissue culture supernatants for 1 h at 4°C followed by addition of a titered amount of goat anti-rat second antibody and incubation on ice for 5 h. The antibody-antigen complexes were washed twice with 20 mM Tris-HCL, pH 7.6 containing 2.5 M KCl, 100 mM NaCl, 1 mM EDTA, 0.5 percent NP-40, and once with 20 mM Tris HCl, pH 7.6. The precipitates were analyzed by SDS-PAGE under reducing conditions (Laemmli, 1970) followed by fluorography (Bonner and Laskey, 1974), unless otherwise indicated. Molecular weight standards were: myosin, Mr 200,000; β-galactosidase, Mr 116,000; phosphorylase B, Mr 97,400; BSA, Mr 68,000; RNA polymerase subunit, Mr 43,000; and chymotrypsin, Mr 23,000.

Light Microscopic Immunohistochemistry

Light microscopic immunohistochemistry was performed as described (McMillan et al., 1981). Tissues obtained from Balb/c (Charles River) or Beige-J (Jackson Laboratory, Bar Harbor, MA) mice, were frozen in liquid nitrogen in OCT mounting compound (Lab-Tek Products Division, Miles Laboratories, Inc., Naperville, IL) on brass chucks. Four µm frozen sections were applied to room-temperature slides precoated with 0.5 percent (w/v) gelatin and 0.05 percent (w/v) chromium potassium sulphate (Fischer Scientific, Pittsburgh, PA). The specimens were immediately fixed in cold acetone for 10 sec, air dried and refrigerated until use. Immediately before

staining, sections were fixed in cold acetone for 3 min, air dried, rinsed in phosphate buffered saline (PBS) for 10 min, and processed as follows: (1) incubated in 0.3 percent $H_2O_2$ in methanol for 10 min at room temperature and rinsed for 10 min in PBS; (2) incubated with 100 ul of a 1:20 dilution of normal human serum (type AB+) in diluent buffer (PBS) containing 3 percent normal human serum for 20 min at room temperature in a humid chamber and then rinsed in PBS for 5 min; (3) incubated overnight at 4° C with 100 ul of a 1:10 dilution of either α-LAMP-1, α-LAMP-2, 5D227 or P3x63Ag8 hybridoma supernatant and washed twice in PBS for 10 min; (4) treated with 100 ul of horseradish peroxidase conjugated goat IgG anti-rat IgG (Kirkegaard and Perry Laboratories, Gaithersburg, MD), 10 ul/ml in diluent buffer, for 30 min at room temperature and washed twice for 10 min with PBS; (5) covered with PBS containing 0.8 ug/ml 3-3'-diaminobenzidine tetrahydrochloride and 0.03 percent $H_2O_2$ for 4 to 5 min, washed with $H_2O$, counterstained with Mayer's hematoxylin for 30 sec, dehydrated, cleared in xylene, and mounted in Permount (Fischer Scientific).

## RESULTS

### Amphiphilic Properties of the Glycoproteins

The association of LAMP-1 and LAMP-2 with lysosomal membranes was compared by use of a variety of detergents and chaotropic agents to extract the glycoproteins into a soluble fraction. As described in Fig. 1, aliquots of cells were incubated with the various extraction reagents and centrifuged at 100,000 x g. The supernatants were retained and the pellets extracted by a lysis buffer containing 0.5 percent Nonidet P-40. Glycoproteins in the supernatant ("supernatant") and extracted pellet ("pellet") fractions were then immunoprecipitated and analyzed by SDS-PAGE (Figure 1).

LAMP-1 and LAMP-2 were each extracted only in the presence of detergent, Triton X-100 or Nonidet P-40. The glycoproteins both remained in the cell pellet when cells were extracted with either 1.0 M KI, 3.0 M guanidine HCl, 1.0 M urea, or 5 mM EDTA. These results indicated that both LAMP-1 and LAMP-2 had properties of amphiphilic molecules with strong hydrophobic associations with lysosomal membranes.

### Biosynthesis of the Glycoproteins

Precursor forms of LAMP-1 and LAMP-2 and processing of the molecules were examined by pulse-chase labeling and immunoprecipitation. HaNIH cells were pulse labeled for 5 minutes with [$^{35}$S]methionine and then incubated with unlabeled methionine for different times. Immunoprecipitates obtained by use of the anti-LAMP-1 or anti-LAMP-2 antibodies were analyzed by SDS-PAGE.

LAMP-1 and LAMP-2 present in the 5 min pulse-labeled fraction (0 chase) and the early chase fractions appeared as multiple forms ranging in apparent molecular weight from about 88,000 to 92,000 (Fig. 2). These precursor forms were processed to mature molecules of approximately 110,000 (LAMP-1) or 105,000 (LAMP-2), beginning at about 30 minutes after pulse-labeling. There consistently was reduced labeling of LAMP-2 by [$^{35}$S]methionine.

Effect of Tunicamycin

Asparagine-linked oligosaccharides are formed via a lipid-linked high-mannose precursor ($Glc_3Man_9GlcNAc_2$) which is transferred co-translationally to nascent peptide chains during their transport across membranes of the rough endoplasmic reticulum (Reviewed by Kornfeld and Kornfeld, 1985). Tunicamycin inhibits the formation of this lipid-linked precursor oligosaccharide thereby preventing N-glycosylation of proteins (Tkacz and Lampen, 1975). Analysis of the effect of tunicamycin may therefore indicate the presence of N-linked oligosaccharides on the mature molecule and reveal the nature of the core polypeptide .

HaNIH cells incubated in the presence of tunicamycin were pulse-labeled with [$^{35}$S]methionine and LAMP-1 and LAMP-2 were immunoprecipitated from detergent extracts of cells. Molecules were present as low Mr core polypeptides, Mr = 42,000 for LAMP-1 (Fig. 3) and Mr = 44,000 for LAMP-2 (data not shown due to the difficulty in photographically reproducing the lightly labeled LAMP-2).

Effect of Endoglycosidase H

The $Glc_3Man_9GlcNAc_2$ oligosaccharides transfered to polypeptides are quickly processed by a series of enzymes present in the rough endoplasmic reticulum and Golgi apparatus, resulting in the removal of the glucose and several mannose residues. For those glycoproteins that traverse the Golgi, this is followed by the addition of other sugars characteristic of complex oligosaccharides. Prior to removal of the mannose residues, the oligosaccharide is sensitive to endo-β-N-acetylglucosaminidase H, which acts specifically on oligosaccharides that contain four or more mannose residues, cleaving the carbohydrate chain between the two proximal GlcNAc residues (Tarentino and Maley, 1979). Treatment with endoglycosidase H should therefore confirm the presence of high mannose oligosaccharides and yield a product that closely resembles the core polypeptide synthesized in the presence of tunicamycin.

HaNIH cells were pulse labeled for 5 min with [$^{35}$S]methionine, LAMP-1 and LAMP-2 were immediately immunoprecipitated from cell extracts, and equal aliquots of the immunoprecipitates were treated with endoglycosidase H or control buffer. The enzyme had a marked effect on both LAMP-1 and LAMP-2: The pulse labeled, unprocessed glycoproteins appeared as single polypeptide bands of approximately 43,000 and 45,000 daltons for LAMP-1 and LAMP-2, respectively (Fig. 4). The untreated, control aliquots contained the expected precursor forms of about 90,000-daltons, as seen in Fig. .

The effect of endoglycosidase H was also studied with cells pulse-labeled and chased for varying times. Post-translational processing of LAMP-1 and LAMP-2 was accompanied by an increase in the apparent molecular weight of the glycoproteins at about 30 min after pulse labeling of the precursor polypeptides, as seen in Fig. 2. These changes can be attributed to the removal of mannose residues from the high mannose core and the addition of N-acetylglucosamine, galactose, fucose and sialic acid residues characteristic of complex oligosaccharides, and possibly the addition of sulfated oligosaccharides. Support for this model was provided by the effect of endoglycosidase H on samples immunoprecipitated at different times after pulse-chase labeling. The precursor glycopeptides remained sensitive to endoglycosidase H after 5, 10, 15, and 20 min of chase incubation following the 5 min pulse-labeling. Both molecules became resistant to the enzyme between 30 and 40 minutes after labeling, corresponding to the time when the proteins showed the apparent increase in molecular weight from the 90,000-dalton precursor glycoproteins to the 105,000 to 110,000-dalton mature forms (D'Souza, unpublished).

Effect of Monensin

Further evidence of the role of the Golgi apparatus in the terminal processing LAMP-1 and LAMP-2 was obtained by use of monensin. Monensin, a monovalent ionophore, markedly effects a number of eukaryotic cell functions,

including the post-translational modification of glycoproteins in the Golgi complex (Tartakoff and Vassalli, 1978; Strauss and Lodish, 1980; Johnson and Spear, 1983).

In the absence of monensin, LAMP-1 and LAMP-2 demonstrated the expected 90,000 molecular weight form at T=0, and were processed at T=120 to the mature forms of 140,000 and 125,000 daltons in P388 and HaNH cells, respectively (Fig. 5). In the presence of monensin, at concentrations as low as 0.2 uM, both LAMP-1 and LAMP-2 at T=120 were both present as diffuse bands of about 85,000 daltons. The same number of acid-precipitable counts were included in each immunoprecipitation reaction, and there was no apparent decrease in the incorporation of $^{35}$S-methionine even at higher doses of monensin, suggesting that there was no significant effect of the drug on the rate of synthesis or metabolic degradation of these antigens. The experiment repeated with HaNH cells gave similar results.

In Vivo Expression of LAMP-1 and LAMP-2

The in vivo expression of LAMP-1 and LAMP-2 was examined by light microscopic immunohistochemistry of tissues from Balb/c and beige-J mice (Figure 6). Beige-J mice have an inherited defect in lysosomal function leading to a Chediak-Higashi-like syndrome (Chi et al., 1978; Frankel et al., 1978). These mice were examined to ascertain whether or not there were any gross alterations in tissue expression of LAMP-1 or LAMP-2 accompanying the Beige phenotype which is characterized by granulation anomalies of leukocytes and gigantism of cytoplasmic organelles (Witkop et al., 1983). LAMP-1 and LAMP-2 displayed essentially identical tissue staining patterns in the two mouse strains. Increased staining of LAMP-1 as compared to LAMP-2 was consistently observed. This was in accord with evidence that LAMP-1 is more abundant than LAMP-2. Both antigens were notably found in macrophages and epithelial cells. Staining was predominantly intracellular. At the light microscopic level we were not able to resolve any differences in cytoplasmic

distribution of LAMP-1 or LAMP-2 in Balb/c as compared to [illegible] cells. The control immunoglobulins in these experiments were the 50287 monoclonal antibody, IgG2a, which reacts with a polymorphic determinant not expressed in Balb/c mice (Hughes and August, 1981) and P3/[illegible] (Kohler et al., 1976). Both gave minimal nonspecific antibody binding.

A principle site of expression of LAMP-1 and LAMP-2 was in epithelial cells. Staining was granular and cytoplasmic. In most simple epithelia, staining was polarized with respect to the cell [illegible]. Strong intracellular staining was observed in the viable epithelium of the tongue surface, apical cytoplasm of epithelial cells of intestinal villi and crypts (Figure 6F), basal cytoplasm of cells lining bronchi and bronchioles, and the efferent ductuli of the epididymis. Kidney tubules contained a mild concentration of these antigens, while glomeruli were negative (Figure 6E). Pancreatic acinar cells were positive in a granular cytoplasmic pattern, while the islets of Langernans were intensely stained (Figure 6D). Staining in hepatocytes was concentrated in a juxtanuclear pattern. Macrophages in most organs, including the lung (Figure 6H), connective tissue, spleen and lymph nodes, stained intensely. Lymphocytes were not stained by either antibody. The antigens were also diffusely located throughout most of the grey and white matter of the brain, with a slight increase of staining in cerebellar Purkinje cells as compared to the granule cell and molecular layers. Other regions of the nervous system were not stained. Staining was absent in stromal tissues, including smooth, cardiac and skeletal muscle, and collagen although there was minor staining in the blood vessel walls.

Expression of LAMP-1 and LAMP-2 in Transformed Cells

There have been reports that lysosomes and lysosomal integrity may be involved in cell transformation (Zajac-Kaye and Tso, 1984). For this reason we have compared the expression of LAMP-1 and LAMP-2 in NIH 3T3 mouse embryo cells and Harvey sarcoma virus transformed NIH 3T3 cells.

The immunofluorescence localization of the antigen was the same in both types of cell, with a vesicular perinuclear staining pattern consistent with that of the lysosomal localization of these antigens (Chen et al., 1985, a,b) (Fig. 7). However, while the pattern of distribution of LAMP-1 and LAMP-2 was indistinguishable between the two cell types, the intensity of staining of LAMP-2 was greater in NIH as compared to HaNIH. This difference between the two cell types was not detected in the intensity of staining of LAMP-1.

## DISCUSSION

In this study we have compared some of the properties of LAMP-1 and LAMP-2, two glycoproteins specifically localized in lysosomal membranes and absent at the plasma membrane or in endocytotic vacuoles. The molecules were markedly similar in many of their properties, particularly in oligosaccharide structure, and in cell and tissue expression. As previously described, the membrane distributions of the molecules analyzed by electron microscopy were identical (Chen et al., 1985a). LAMP-1 was a major component of the membrane; it constituted 0.1 percent of total detergent extracted cell protein and, when labeled with [$^3$H]glucosamine, the immunoprecipitated glycoprotein accounted for about 15 percent of the total acid-insoluble radioactivity fractionated by two-dimensional gel electrophoresis (Hughes and August, 1981). The concentration of LAMP-2 has not been determined but the antigenic reactivity of the molecule and the incorporation of sugar substrates were comparable to that found with LAMP-1. Biosynthetic labeling of LAMP-2 with [$^{35}$S]methionine was markedly less than that of LAMP-1, but this could be attributed either to the concentration of the protein, methionine content, or rate of synthesis. Glycoproteins with similar properties were described by Burnside and Schneider (1982) as major components of lysosomal membranes and by Kato et al. (1984) as constituting 5 percent of the total protein and 10 percent of the membrane protein of tritosomes. The tissue expression of the molecules in the mouse, including the Beige-J mouse genetically deficient in lysosomal function, also appeared to be identical. Both proteins were present in cells known to contain high concentrations of lysosomes. In addition, intense staining of pancreatic islet cells and a granular pattern of staining in acinar cells suggested that both glycoproteins were also components of secretory and storage granules. Possible evidence for independent expression of LAMP-1 and LAMP-2 was obtained in comparing the immunohistochemical staining of the protein in different cell lines. In repeated experiments the

intensity of immunofluorescent labeling of LAMP-2 in Harvey sarcoma virus transformed NIH 3T3 cells was reduced as compared to the untransformed cells, whereas there was no difference in LAMP-1 expression between the two cells.

Distinctive features of LAMP-1 and LAMP-2 were the high concentrations of oligosaccharides. A model suggested by the data is that the glycoproteins contain a large number of asparagine-linked high mannose oligosaccharides added cotranslationally and then processed to chain sequences found in complex-type oligosaccharides. Studies with tunicamycin, which blocks assembly of the lipid-linked oligosaccharide precursor, indicated that the core polypeptides of LAMP-1 and LAMP-2 were synthesized as molecules of 42,000 and 44,000 daltons, respectively. These results were substantiated by the effect of endoglycosidase H, which acts specifically on high mannose oligosaccharides. The approximately 92,000 dalton precursor glycoproteins pulse-labeled with [$^{35}$S]methionine for 5 min and isolated by immunoprecipitation were converted by the enzyme to polypeptide bands of 43,000 and 45,000 daltons for LAMP-1 and LAMP-2, respectively. The results also indicate that the multiple bands of the precursor glycoproteins found by SDS-PAGE analysis of the unprocessed, pulse-labeled samples can be attributed to heterogeneity in the asparagine linked high mannose core oligosaccharides of these glycoproteins. The core polypeptides obtained after treatment with tunicamycin or endoglycosidase H were present as single bands. The difference in apparent molecular weight between the approximately 43,000-dalton core and 92,000 dalton precursor molecules would be sufficient for as many as 20 to 25 high mannose chains per polypeptide. Lewis et al. (1985) directly demonstrated at least 18 asparagine-linked oligosaccharides on the lpg120. Subsequent processing of the mannose-rich precursor to mature molecules of 110,000 and 105,000 for LAMP-1 and LAMP-2, respectively, can be attributed to the formation of complex-type, highly sialiated oligosaccharides. The 5 min [$^{35}$S]methionine pulse-labeled molecules became resistant to endoglycosidase

H after about 30 min of chase incubation, corresponding to the appearance of the mature glycoproteins. Simultaneously, the molecules became highly sialiated, with a large number of acidic isoelectric variants revealed by two-dimensional gel electrophoresis, particularly with LAMP-1 which contained more than 16 distinct fractions between pH 4.1 and 7.0 (Chen et al., 1985a). Neuraminidase eliminated these acidic forms, yielding molecules with an alkaline pH (Chen, et al., 1985b). The basis for the apparent increase in molecular weight during terminal processing is unknown. The molecules were markedly heterogeneous in that the apparent molecular weight of [$^{3}$H]glucosamine-labeled glycoprotein appeared greater than that of [$^{35}$S]methionine-labeled molecules or protein stained with either the Coomassie Blue (Chen et al., 1985a). It thus appeared that a small fraction of the protein contained a majority of the sugar residues and that this heavily glycosylated fraction correspondingly migrated at higher apparent molecular weights. The effect was not attributed to sialic acid as there was no comparable effect of neuraminidase on molecular weight. Treatment with monensin blocked the increase in apparent molecular weight and resulted in molecules of about 85,000 daltons, slightly smaller than the normal pulse-labeled precursor of about 92,000 daltons. Ongoing studies (Mane, unpublished) show that monensin markedly reduces the rate of processing of the high mannose oligosaccharides and it is speculated that some process occurring during terminal processing in the Golgi and related to the apparent change in molecular weight was blocked by the drug. For example, the reduced rate of migration in SDS-PAGE electrophoresis of the low-density lipoprotein receptor has been related to O-linked residues (Cummings, et al., 1983) and it is possible that there is O-linked glycosylation of the LAMP proteins in the Golgi.

These results provide evidence that the biosynthesis of both LAMP-1 and LAMP-2 involve passage through the Golgi apparatus and processing of the

N-linked high mannose chains to complex-type oligosaccharide. This likely differs from the processing of lysosomal enzymes which do not appear to traverse the Golgi system and are modified by a highly specific mannose-6-phosphate recognition system catalyzed by enzymes believed to reside in the cis Golgi cisternae reviewed by Kornfeld and Kornfeld, 1985). In repeated attempts, we have not detected phosphorylation of the LAMP glycoproteins. Moreover, we recently have identified a molecule in human cells that is homologous to LAMP-2 and find this glycoprotein markedly enriched in cells derived from patients with I-cell disease (MLII) or pseudo-Hurler polydystrophy (MLIII) (Chen, unpublished). These diseases are characterized by the absence of the mannose phosphate recognition marker on lysosomal enzymes and diminished cellular concentrations of the enzymes.

The recently described lysosomal membrane glycoproteins are similar in several properties. One remarkable correlation is the high content of N-linked oligosaccharide. The rat protein lpg120 of Lewis et al., (1985), the chicken CV24 antigen of Lippencott-Schwartz and Fambrough, and LAMP-1 and LAMP-2 all contain polypeptide cores of 40,000 to 50,000 daltons that are modified by high mannose oligosaccharides to precursor glycoproteins of about 90,000 daltons and processed to mature glycoproteins of about 110,000 to 120,000 daltons. It can be speculated that this high carbohydrate composition is important to the function of the glycoproteins. Another relationship is the presence of CV24 protein and the 100,000 dalton glycoprotein identified by Reggio (1984) on endosome and plasma membranes in addition to the lysosomal localization, whereas the lpg120 Lewis et al., (1985) and LAMP-1 and LAMP-2 are restricted to lysosomes. Moreover, the Reggio antigen and CV24 were found on the ruffled border plasmalemma of osteoclasts whereas the lpg120 was absent (Baron et al., 1985). It is likely that some of these glycoproteins are homologous. Unfortunately, direct immunological comparison is difficult because of the different species of origin of the antigens. Definitive comparison of the proteins awaits peptide sequencing.

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FIGURE LEGENDS

Fig. 1 LAMP-1 and LAMP-2 are integral membrane proteins. HaNIH cells were metabolically labeled with [$^{35}$S]methionine as described in Materials and Methods. The washed cells were aliquoted into seven pre-weighed conical tubes and collected by centrifugation. Various extraction agents were added to the pellets at a ratio of extraction agent:cell pellet=10:1 (w/w), as follows: A, Hank's buffered saline; B, 1.0 percent Triton-X-100; C, 5 mM EDTA with 3 cycles of freeze-thaw; D, 1.0 M KI; E, 50 mM guanidine HCL; F, 1.0 M urea; and G, 0.5 percent Nonidet P-40. To each of these PMSF was added to a final concentration of 1mM. After incubation at room temperature for 30 min the cells were centrifuged at 100,000 x g for 1 hr at 4°C. The supernatants were dialyzed exhaustively against lysis buffer (0.5 percent Nonidet P-40, 5 mM EDTA, 150 mM NaCl, 10 mM Tris-HCl, pH 7.5) and centrifuged at 100,000 x g for 1 hr at 4°C ("Supernatant"). The pellets from the first 100,000 x g centrifugation were extracted a second time with lysis buffer and the suspension was centrifuged at 100,000 x g. The supernatant was retained ("pellet"). 1 x $10^6$ acid precipitable dpm of the supernatant and the pellet extracts were incubated with the α-LAMP-1 or α-LAMP-2 monoclonal antibodies and the immune complexes were processed and analyzed by SDS-PAGE as described in Materials and Methods.

Fig. 2 Biosynthetic processing of LAMP-1 and LAMP-2 in cells pulse-labeled with [$^{35}$S]methionine. HaNIH cells were pulse-labeled for 5 min, chased for the indicated time, and extracted and analyzed by immunoprecipitation with anti-LAMP-1 and anti-LAMP-2 monoclonal antibodies followed by SDS-PAGE, as described in Materials and Methods.

Fig. 3 Identification of a core precursor polypeptide in cells treated with tunicamycin. HaNIH cells were preincubated and pulse-labeled for 15 min with [$^{35}$S]methionine in the presence (tunic) or absence (control) of 2 ug/ml tunicamycin (Calbiochem, La Jolla, CA). The cells were extracted and $10^6$ dpm of acid-precipitable radioactivity were incubated with anti-LAMP-1 and the immunoprecipitates were analyzed by SDS-PAGE as described in Materials and Methods.

Fig. 4 Identification of the products of pulse-labeled LAMP-1 and LAMP-2 treated with endoglycosidase H. HaNIH cells labeled with [$^{35}$S]methionine for 5 min were harvested immediately. The immune complexes obtained with anti-LAMP-1 and anti-LAMP-2 as described in Materials and Methods were solubilized in 40 ul of Endo H buffer (0.1 M Na citrate, pH 5.6, 0.1 percent SDS, 0.2 percent B-mercaptoethanol, and 1 mM PMSF) by boiling for 2 min. Immunoprecipitates were divided into two equal aliquots. Two milliunits of endoglycosidase H (Boeringher Mannheim, Indianapolis, IN) were added to one sample and both were incubated at 37°C for 12 hrs. The proteins were analyzed by SDS-PAGE followed by fluorography.

Fig. 5 Monensin effects the post-translational processing of LAMP-1 and LAMP-2. HaNIH and P388 cells were pulse-labeled with [$^{35}$S]methionine for 5 min and incubated in the presence of unlabeled methionine as described in the text with the exception that 0.2 to 10.0 μm monensin (Calbiochem) was included in the preincubation, labeling, and chase media. A and B: LAMP-1. C and D: LAMP-2. A and C: HaNIH cells. B and D: P388 cells. Control cells without monensin were pulse-labeled for 5 min and harvested immediately (lanes

1) or chased for 120 min (lanes 2). Other cells were all preincubated, labeled for 5 min and chased for 120 min in the presence of different concentrations of monensin: 0.2 uM, (lanes 3); 0.5 uM, (lanes 4); 1.0 uM, (lanes 5); 2.0 uM, (lanes 6); 5.0 uM, (lanes 7). The cells were extracted, immunoprecipitated and examined by SDS-PAGE.

Fig. 6 In vivo expression of LAMP-1 and LAMP-2. Frozen sections of mouse tissue were incubated with anti-LAMP-1 (f,h), anti-LAMP-2 (b,d), ED227 (e,g), or P3x63A8 (a,c), and then processed as described in Materials and methods. For each tissue examined, the sections treated with control antibodies were devoid of reaction product (a,c,e,g). Kidney of Beige-J mouse (a,b): Tubules (T) stained intensely while glomeruli (G) show negligible staining. Pancreas from Beige-J mouse (c,d): The pancreatic acinar (pa) cells show moderate granular cytoplasmic staining, while the islets of Langerhans (I) stained intensely. Large intestine from Balb/c mouse (e,f): Staining of epithelial cells of intestinal villi and crypts appeared to be concentrated in the apices of the cells. Lung from Balb/c mouse (g,h): Intense intracellular and surface staining of alveolar macrophages (M). Magnification = x1000.

Fig. 7 Immunocytochemical localization of LAMP-1 and LAMP-2 in NIH and HaNIH cells. NIH (A,B) and HaNIH (C,D) cells were grown to 50 percent confluency in 35-mm plastic dishes and fixed in situ with 3.0 percent paraformaldehyde in PBS for 10 min. The cells were washed several times with PBS followed by Dulbecco's modified Eagle's medium. They were then incubated for 30 min at room temperature with anti-LAMP-1 (A,C) or anti-LAMP-2 (B,D) culture supernatant diluted 1:10 with PBS, 0.1 percent BSA, and 0.1 percent saponin (Sigma, St. Louis, MO). The cells were then washed with PBS and incubated for an additional 30 min

in PBS, 0.1 percent BSA and with affinity-purified goat anti-rat IgG conjugated to rhodamine (Kirkegard and Perry) in PBS, 0.1 percent BSA and 0.1 percent saponin. Cells were photographed using a Zeiss microscope equipped with epiilumination and a 100X, N.A. 1.4 oil objective. Photographs were made with Kodak Ektachrome P800 film and processed by E-6P procedure. All photographs were made at the same exposure. N=-nucleus. Mag = X 1200.

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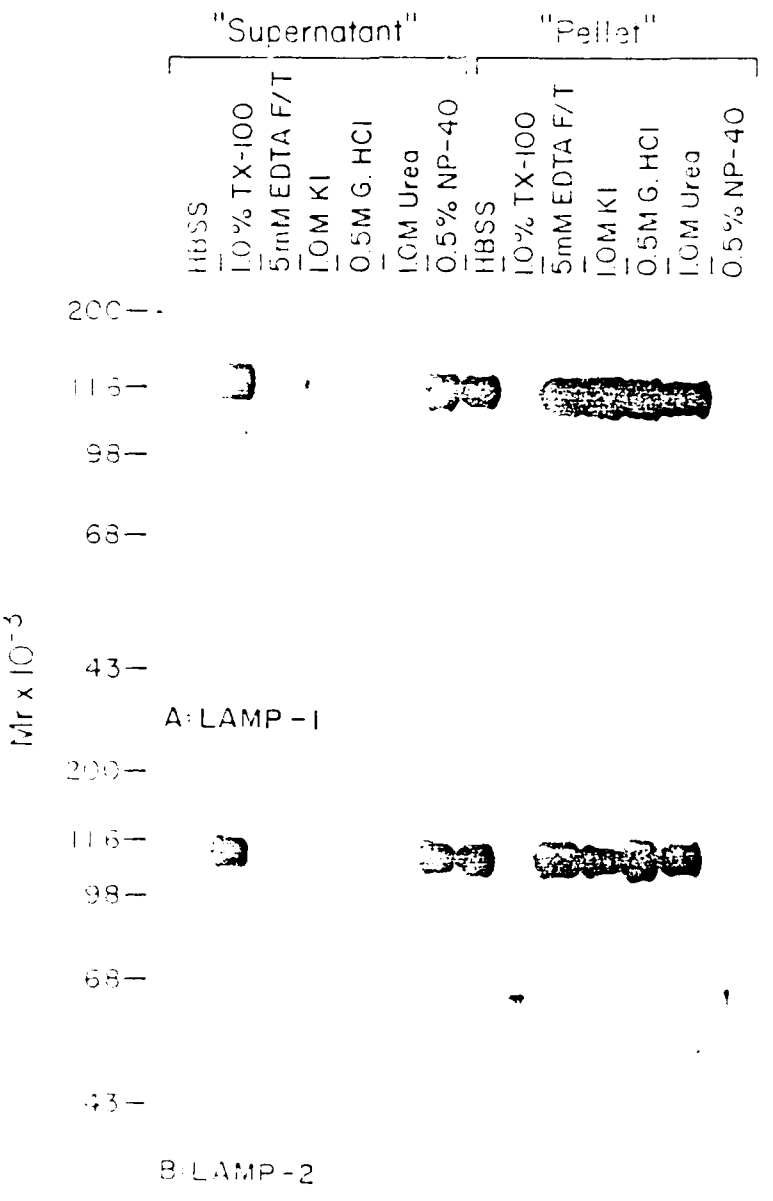
FIGURE 1


"Supernatant"
"Pellet"
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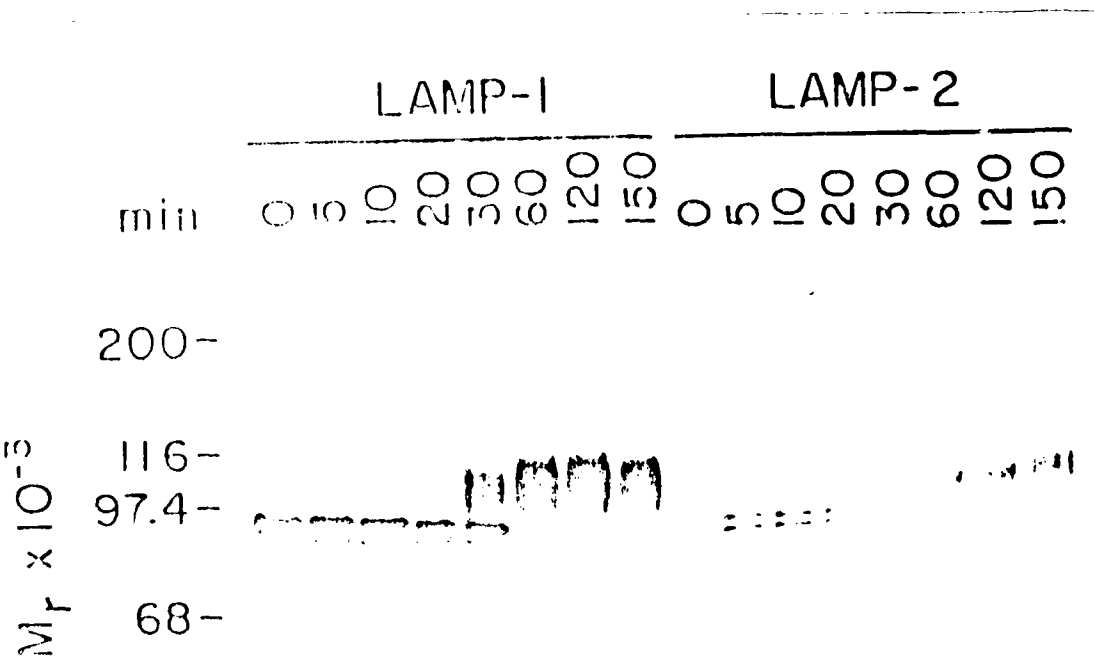
5mM EDTA F/T
1.0M KI
0.5M G. HCl
1.0M Urea
0.5% NP-40
HBSS
1.0% TX-100
5mM EDTA F/T
1.0M KI
0.5M G. HCl
1.0M Urea
0.5% NP-40
200
116
98
68
43
Mr x 10⁻³
A: LAMP-1
200
116
98
68
43
B: LAMP-2

FIGURE 2


LAMP-1
LAMP-2
min
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0 5 10 20 30 60 120 150
200-
116-
97.4-
68-
43-
$M_r \times 10^{-3}$

FIGURE 3

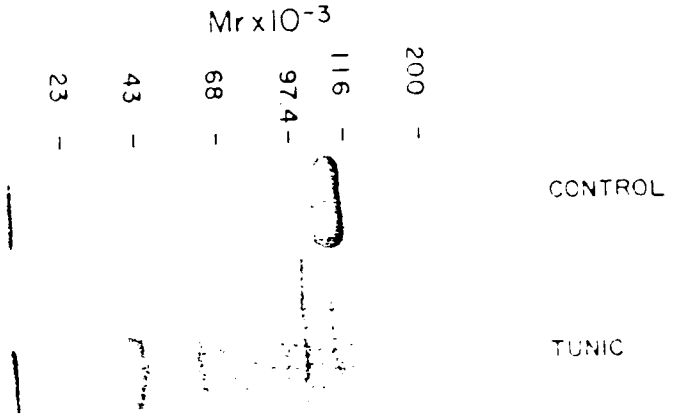


FIGURE 4

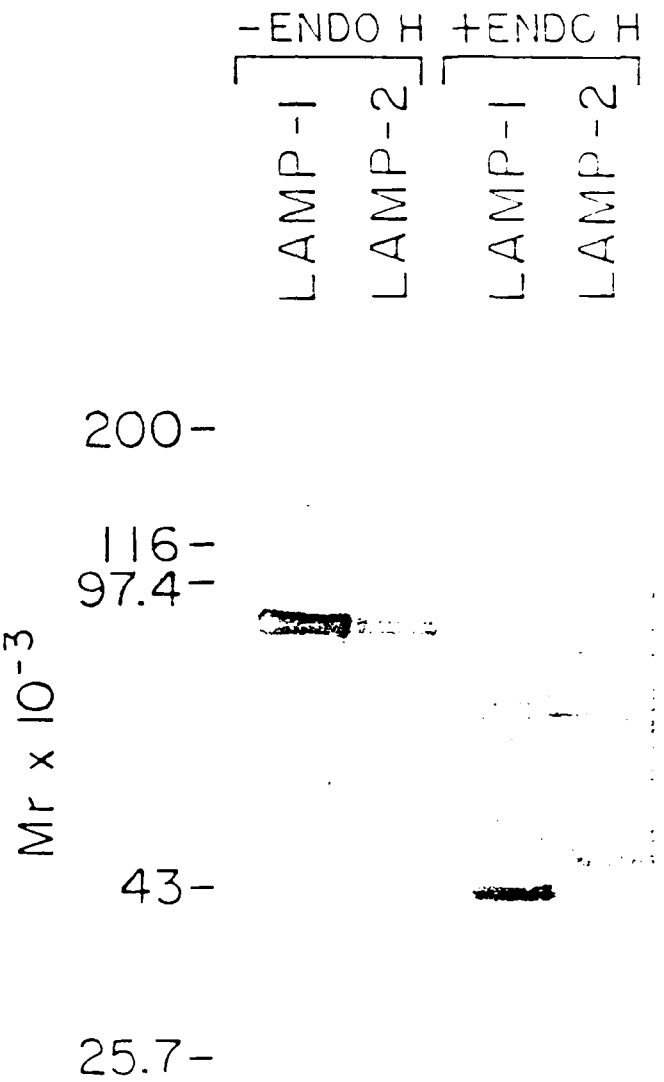
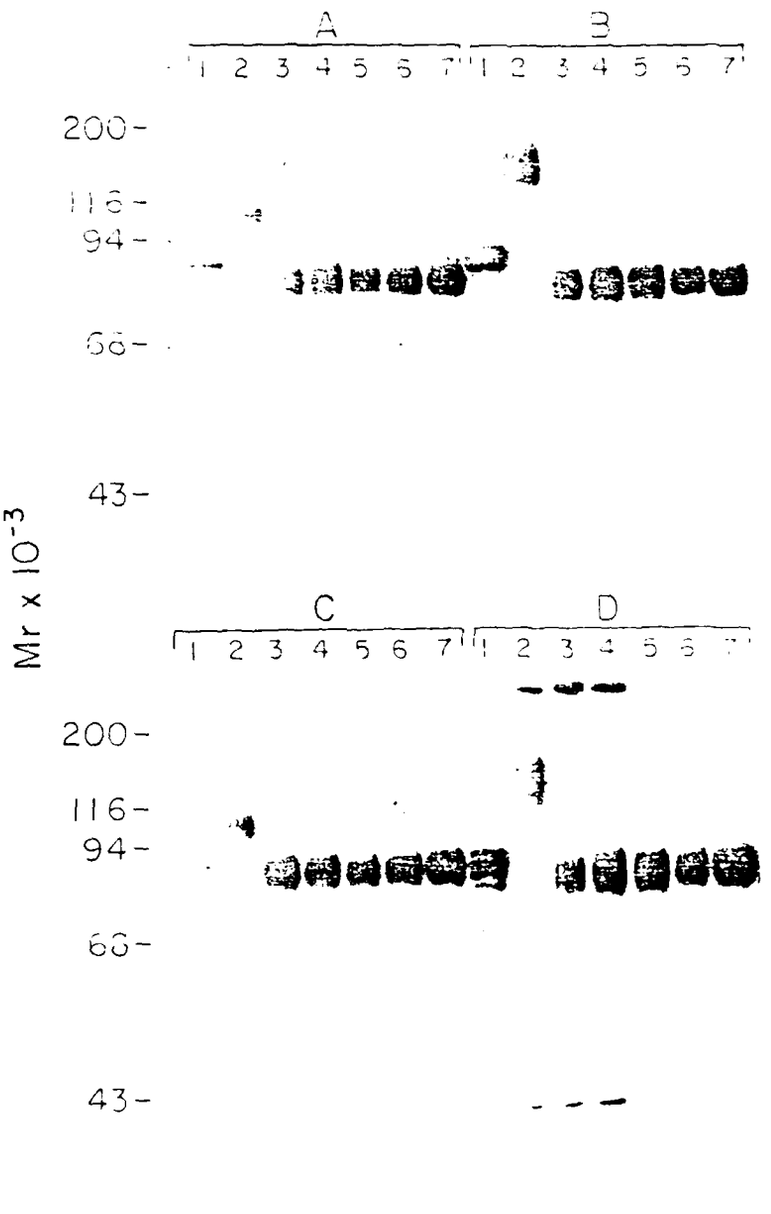

-ENDO H
+ENDO H
LAMP-1
LAMP-2
LAMP-1
LAMP-2
200-
116-
97.4-
43-
25.7-
Mr x 10-3

FIGURE 5


A
B
1 2 3 4 5 6 7 1 2 3 4 5 6 7
200-
116-
94-
66-
43-
Mr x $10^{-3}$
C
D
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200-
116-
94-
66-
43-

FIGURE 6

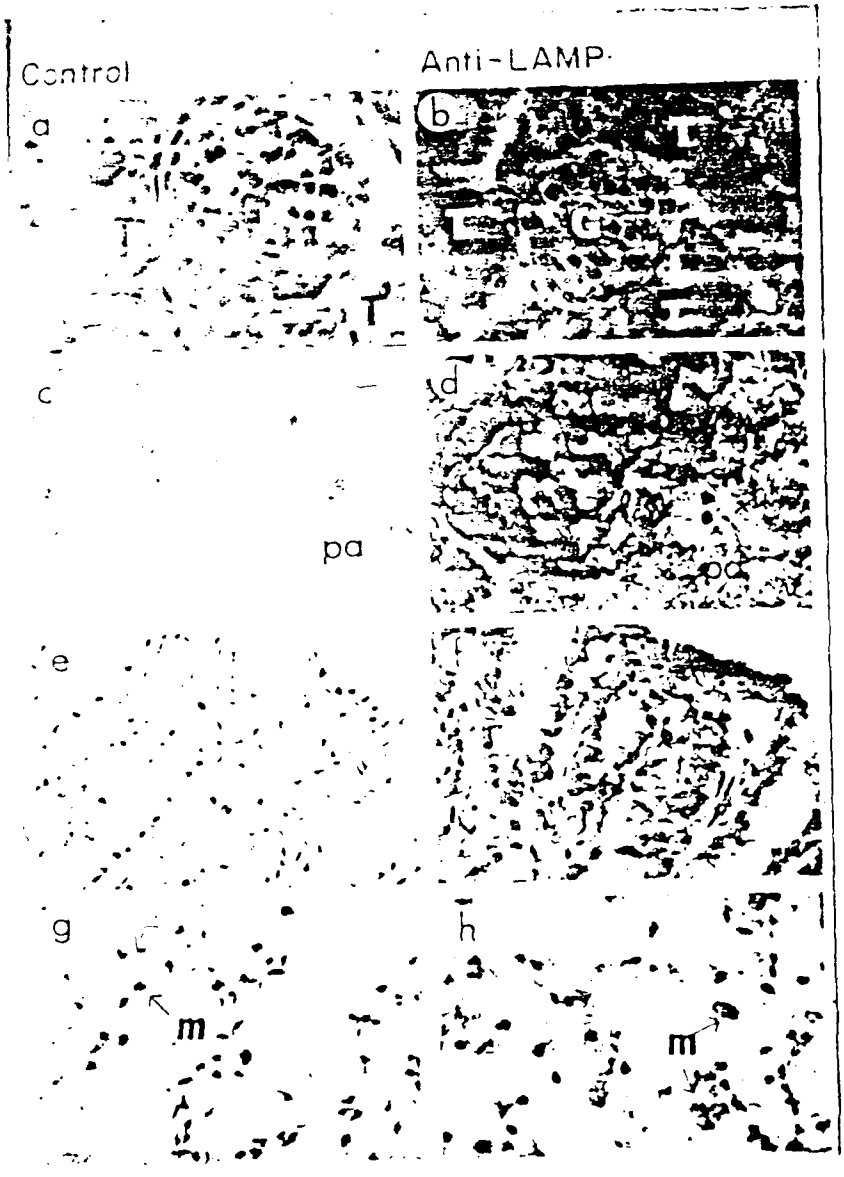

FIGURE 7